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Award Number: DAMD17-00-1-0384

TITLE: Solid Phase Combinatorial Approach to Estradiol Tamoxifen/Raloxifene Hybrids: Novel Chemotherapeutic/Prophylactic Selective Estrogen Receptor Modulators

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REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 2002 | Annual (1 Jul 01 -30 Jun 02) |

**4. TITLE AND SUBTITLE**
Solid Phase Combinatorial Approach to Estradiol Tamoxifen/Raloxifene Hybrids: Novel Chemotherapeutic/Prophylactic Selective Estrogen Receptor Modulators

**5. FUNDING NUMBERS**
DAMD17-00-1-0384

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. Abstract** *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

The objective of this project is the development of new chemotherapeutic agents for the treatment of hormone-responsive breast cancer using a solid phase approach to synthesize new agents having features common to both steroids and antiestrogens.
Previously we functionalized the carboxy resin with both the E-and Z-tributylstannylvinyl estradiol, and prepared an initial series of iodophenoxyalkylamines that will be coupled to the resin-bound steroid. Coupling reactions with the Z-stannylvinyl estradiol were generally unsuccessful on solid-phase and coupling with the E-isomers proceeded in low yields. We have prepared more iodophenoxyalkylamines and are preparing the target compounds via solution phase methods. We are exploring an approach using resin-bound estradiol vinylboronic acids as an alternative method.

**14. SUBJECT TERMS**
breast cancer

**15. NUMBER OF PAGES**
13

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

## Table of Contents

## 4. Introduction.

The overall objective of this project is the development of new chemotherapeutic agents for the treatment or prevention of hormone-responsive breast cancer. Our approach involves the solid-phase synthesis of a series of 17α-(substituted-phenyl)vinyl estradiols in which the substituent is derived from the anti-estrogen imparting components of tamoxifen and raloxifene. The new compounds would be evaluated by appropriate biological assays to determine the receptor binding affinity and efficacy. The results would be evaluated to determine the targets for subsequent synthetic efforts designed to enhance the biological properties of the substances. This report describes the efforts made during the past year to achieve those objectives.

## 5. Body

The research proposal described 5 specific tasks in the Statement of Work. These were: 1. Initial target compound design. 2. Chemical synthesis of target compounds in initial directed library. 3. Measurement of biological properties (receptor affinity and efficacy). 4. Assessment of structure-activity relationships. 5. Chemical synthesis of target compounds in second generation libraries. The completion of the first task was described in the report last year. Work on the second and third tasks continued during this past year and will be described in this report.

### Task 2. Chemical synthesis of target compounds in initial directed library. (Months 1-24).

During this period we focused on two aspects. The first was continued preparation of the series of dialkylaminoalkoxylphenyl iodides that constitute the coupling partners for the solid phase Stille reaction. The second was the synthesis of the target compounds on solid phase followed by cleavage, purification and characterization.

The synthesis of virtually all of the dialkylaminoalkoxyphenyl iodides in the ethoxy- and propoxy series has been completed. The ethoxy- series was achieved in good yields (75-85%) in one step from the commercially available hydroxyethyl amines and the iodophenols using the Mitsunobu reaction. The propoxy-series was prepared in two steps from bromopropanol and the iodophenol (Mitsunobu reaction) followed by reaction with the appropriate dialkyl amine. Overall yields were lower (50%) but still satisfactory. Preparation of the butoxy-series is in progress using the second method. The products, as their oxalate salts, are available for the subsequent coupling reaction.

The Stille coupling of the iodophenyl ethers and the resin-bound E- and Z-tri-butylstannylvinyl estradiols was undertaken using the procedure employed for the synthesis of the simpler substituted phenylvinyl estradiols. Reactions with the E-isomer gave low yields of product along with a mixture of by-products. The reactions were repeated without being able to significantly improve the yields. Sufficient quantities of the dimethylaminoethoxyphenyl-vinyl estradiol were obtained to submit for biological evaluation. Reactions with the Z-isomer gave no characterized product. This observation was similar to what we had obtained with some of the solution couplings with the Z-isomer.

In order to obtain sufficient material in the target series we have temporarily reverted to the solution based chemistry. We are concentrating on the E-isomers because they can be obtained more reliably, in higher yield and they are chemically more stable. We are also exploring the use of the Suzuki coupling reaction and so have done preliminary work in preparation and coupling of vinyl boronic acids. In order to preserve the more valuable ethynyl estradiol starting material, we have used a simpler estrogenic core [3,5-bis-(4-hydroxyphenyl)-isoxazole] described by Katzenellenbogen, as a model system. We have been able to prepare phenyl vinyl derivatives via two approaches using this scaffold and are now applying this methodology to the ethynyl estradiol series. We have started to prepare the estradiol vinylboronic acids and esters in preparation for both the Suzuki solution and solid phase organic syntheses. While the initial work will be done using solution chemistry, we will keep in mind the application to solid phase organic synthesis.

**Task 3. Measurement of biological properties-affinity and efficacy (Months 1-24).**

We have continued to develop the biological evaluative methods for the new compounds. As described in the first report we have established the assays for determining the receptor binding affinity utilizing the ligand binding domain overexpressed in a bacterial cell line. The initial evaluation was with the ER-alpha-LBD, although we have been able to extend this to the ER-beta-LBD as well. We

used these two ER-LBDs to evaluate the model isoxazoles prepared as part of our boronic acid study. We also have evaluated the first of the dialkylaminoalkoxyphenylvinyl estradiols to begin the comparison of the target compounds versus the simpler phenylvinyl estradiols.

We have also started the evaluation of the isomeric E-/Z-substituted phenylvinyl estradiols (6 compounds per series) in the immature female rat uterotrophic growth assay. Such assays involve 280 rats per study in order to be able to do a direct comparison of the compounds. We had found that we could not obtain the same results by pooling data from separate assays. In these recent assays, we have observed that the uterotrophic data do not always correspond to the binding data. So far, for the 5 series that we have evaluated, the ortho-substituted phenyl vinyl compounds (both E- and Z-isomers) usually are the most active. Also, the simple substituted phenylvinyl compounds are all agonists (estrogenic). Therefore, as we proceed to the dialkylaminoalkoxyphenyl vinyl series, we hope to observe a transformation to antagonist (anti-estrogenic) properties.

To enhance our ability to assess both affinity and efficacy we are starting to generate the stably transfected ERα/β-LBDluciferase assay. This will allow us to determine simultaneously the affinity and efficacy of the new compounds much more rapidly than currently possible.

**Task 4. Assessment of structure-activity relationships (Months 6-24).**

We have started to develop the structure-activity relationships for the 17α-(substituted-phenyl)vinyl estradiols. In conjunction with the other projects we have undertaken the molecular modeling docking studies with the ligands and the ER-LBD. Our initial molecular dynamics docking studies with the para-substituted phenyl vinyl estradiols gave a linear relationship between the calculated binding energies and the relative binding affinities (RBA). The studies also suggest that the

region into which we are introducing the dialkylaminoalkoxy-side chains should be able to accommodate the substituent.

The evaluation of the in vivo data suggests that the simpler derivatives are full agonists with potencies ranging from more active than estradiol to less than 1% as potent as estradiol. In most, but not all cases, the ortho-isomer in both the E- and Z-series is the most active. In the E- series, the meta- and para-isomers are generally, but not always, weak estrogenic agonists. In the Z-isomers, the meta- and para-isomers are quite active, but not as potent as the ortho-products.

## 6. Research Accomplishments.

- Completed preparation of most dialkylaminoalkoxyphenyl iodide coupling reagents
- Developed molecular dynamics methods for evaluating ligand binding energies and RBA
- Developed in vivo uterotrophic assay and in vitro transfection luciferase assay
- Synthesized phenylvinyl derivatives of diaryl isoxazoles as models for alternate boronic acid approach
- Completed initial SAR studies for simple para-substituted phenylvinyl estradiols

## 7. Reportable Outcomes.

a. Manuscripts, abstracts, presentations

1. Evaluation of 17α-(X-phenyl)vinyl estradiols as estrogen receptor agonists. Robert N. Hanson, Carolyn Friel, Choon Young Lee, Robert Dilis, Eugene R. DeSombre, Alun Hughes. Medicinal Chemistry Gordon Conference, New London, NH. August 4-9, 2002. Poster.
2. Evaluation of 17α-(X-phenyl)vinyl estradiols as estrogen receptor agonists. Robert N. Hanson, Carolyn Friel, Choon Young Lee, Robert Dilis, Eugene R. DeSombre, Alun Hughes. 224 ACS National Meeting, Boston, MA. August 18-22, 2002. Poster MEDI 359.
3. Mitsunobu Reaction: A versatile synthetic and educational tool. Robert N. Hanson, Katharine M. Gray and Michael Bianchi. 224 ACS National Meeting, Boston, MA. August 18-22, 2002. Poster CHED 197.
4. Synthesis of 4-substituted-3,5-diarylisoxazoles by palladium-catalyzed coupling reactions. Rachel E. Gershman, Eugene R. DeSombre, Robert N. Hanson and Alun Hughes. 224 National ACS Meeting, Boston, MA. August 18-22, 2002. Poster MEDI 385.
5. Several manuscripts are in progress in which the material presented in the posters will be described in greater detail.

b. Degrees obtained supported by the award.

1. Rachel E. Gershman, Synthesis of 4-(Substituted Phenylvinyl)-3,5-diaryl-isoxazoles. Approaches to combinatorial libraries via Suzuki coupling reactions. M.S. in Chemistry, Fall 2002.

## 8. Conclusions.

At this point, we are continuing to make progress on completing our ultimate objectives. We have had difficulty translating our initial success in synthesizing simpler estrogens on solid phase to the preparation of more complex compounds. We have continued to prepare the key reagents and develop alternatives, including solution based syntheses. We have expanded our biological assays to include in vivo uterotrophic growth assays and an in vitro transfection assay. Preliminary biological results indicate that simpler estrogenic derivatives retain full receptor potency. Molecular dynamics studies demonstrate a direct relationship between calculated binding energies and observed binding affinities. For the next year we will continue to prepare the initial series of target compounds and evaluate their estrogen receptor-related properties.

## 9. References.

None.

## 10. Appendix.

The appendix material consists of copies of the 3 posters for the presentations at the Gordon Conference and at the ACS meeting.

# Preparation and Evaluation of Isomeric series of 17α-(Substituted-phenyl)vinyl Estradiols

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**Introduction:**

The estrogen receptor (ER) is a member of the superfamily of nuclear receptors (NR)[1]. This superfamily is characterized by a common structural homology consisting of six domain- the N-terminal Activation Factor-1 domain, a DNA binding domain (DBD), a hinge region, a hormone binding domain (HBD), and a carboxy-terminal Activation Factor-2 domain. Studies have shown that the NR mechanism of action proceeds via similar pathways, but is initiated by the binding of the endogenous hormone to the HBD [2,3]. Because the estrogen receptor is implicated with many normal biological functions, including sexual development, cardiovascular and bone physiology, as well as lipid metabolism, significant interest has been devoted to identifying the role that initial ligand binding plays in mediating the hormonal processes. In addition, the association of elevated ER levels with hormone responsive breast cancer has stimulated research into blocking the deleterious effects of estrogens in cancer cells while maintaining the beneficial effects elsewhere.

As part of our program to develop novel probes for the estrogen receptor, we selected as our lead compounds the isomeric 17α-E- and Z-phenylvinyl estradiols. We had prepared these and similar compounds as potential imaging agents and found that the compounds had retained significant affinity for the ER. Subsequent publications of the crystal structures [4,5] of a variety of ligands with the ER(α/β)-HBD suggested to us that the 17α-substituent may be accommodated in the peripheral region of the binding site and that the use of E/Z-isomers as well as ortho-, meta-, and para-substitution may provide a means for probing that region (Figure 1). The benefits of such probes would be: 1.To understand the interactions between the ligand and the receptor, 2. To develop high affinity ligands; 3. To identify ER-subtype selective compounds; and 4. To develop ligands that would interfere with the activation process. This presentation describes the results of our biological evaluations of the series of compounds prepared to probe the ERα-HBD.

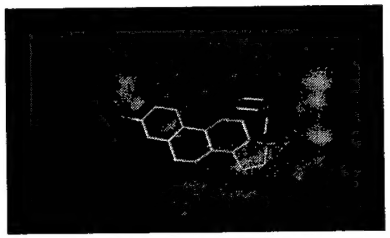
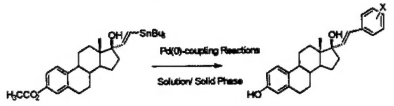


Figure 1

**Synthesis:**

The target compounds were prepared utilizing methods developed in our laboratory. The 17α-E- and Z-tri-n-butylstannylvinyl estradiols were converted to the corresponding 17α-E/Z-(2-/3-/4-substituted-phenyl)vinyl estradiol 3-acetates and hydrolyzed to the final estradiol derivatives. Although the compounds could be obtained via a conventional Stille coupling, several were prepared by alternate processes to demonstrate the feasibility of solid-phase and Suzuki approaches [6]. All compounds were characterized by $^1$H- and $^{13}$C-NMR, and by elemental analysis.




Receptor Binding:

The compounds were screened for their affinity for the ERα-LBD isolated from BL 21 cells that over-expressed the 33kDa PER-23d ERG vector [7]. The cells were induced with 0.6 mM isopropyl-β-thiogalactopyranoside for 3h at RT, pelleted by centrifugation, frozen and stored at −75 °C. The cells were thawed, and lysed by sonication (4X20 sec) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4) several times. Clarified fractions, obtained at 30,000 x g for 30 min were pooled, assayed for receptor binding, diluted to 50 nM in ER and 100 µL aliquots were frozen and stored at −75 °C until ready for use. Then 80 µL of the ERα-LBD- containing extract was incubated with 10 µL of 10 nM 6,7-[H-3]-estradiol (specific activity = 51 Ci/mmole) and 10 µL of either buffer, unlabeled estradiol or test ligand in 100 µL total volume. The final concentrations were 1 nM 6,7-[H-3]-estradiol, 2 nM unlabeled estradiol, (using 200 nM estradiol to define specific binding) and 0.5-5000 nM of the test ligand. In all cases, 10 µL of each incubation solution was removed for assay of the actual initial concentration of [H-3]-estradiol and the remainder was incubated at 2 °C or 25° C for 18 hours. After incubation, 100 µL of dextran coated charcoal suspension (fines removed) was added to adsorb the unbound [H-3]-estradiol, incubated for 10 min, centrifuged , and 100 uL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as % specific binding as a function of log of competitor concentration using the best fit equation for the binding inhibition to define 50% inhibition level. The relative binding affinity (RBA) was calculated as 100 times [E]/[C], where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]-estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%. The results for the target compounds are shown in Table 1.

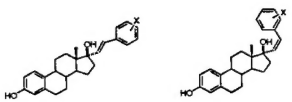



Figure 2

| X= | Ortho- | Meta- | Para- | Ortho- | Meta- | Para- |
|---|---|---|---|---|---|---|
| F | 15 | 20 | 37 | 73 | 16 | 33 |
| $CF_3$ | 223 | 75 | 8 | 26 | 60 | 9 |
| $CH_3$ | 27 | 12 | 18 | 27 | 45 | 9 |
| $CO_2CH_3$ | 23 | 26 | 17 | 49 | 12 | 57 |
| OH | 46 | 91 | 25 | 57 | | 25 |
| H | | 15 | | | 66 | |
| Estradiol | 100 | | | | | |

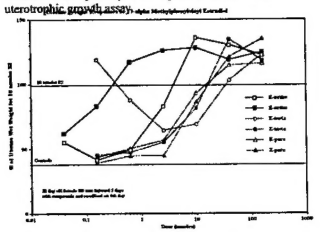
**Table 1. Comparison of Relative Binding Affinities (RBAs) for Compounds in Study**

**Uterotrophic Growth Assay:**

Test ligands were evaluated using the uterotrophic growth assay [8]. Groups of immature female rats (at least 5 per group) were injected subcutaneously starting with either vehicle (control), 1, 10,or 100 nmoles of test ligand in 0.1 mL saline (0.5%) and the uterine weights were compared to that of rats receiving 0.3, 1.0, 3.0 and 10 nmole of estradiol for 3 days. Animals were sacrificed 24h after the last injection, uteri were removed, stripped free of fat and connective tissue, weighed wet, dried in vacuo and weighed to dry weight. Curves of uterine weight (wet and dry) vs. amount of compound injected were compared to assess the potency of the test compound vs. estradiol. Potency for the test ligands was calculated based on the nmole dose equal to 50% of maximal stimulation for the ligand ($ED_{50\%max}$). The results are given in Table 2 and illustrated by typical curves Figures 2-3. The absence of antagonist effectsisshown in Figure 4.

| Substituent | Z-Isomer $EC_{50\%max}$ | | E- Isomer $EC_{50\%max}$ | |
|---|---|---|---|---|
| $CH_3$ | Ortho | 0.16 | Ortho | 3.0 |
| | Meta | 11.5 | Meta | 8.8 |
| | Para | 15.4 | Para | 5.4 |
| $CF_3$ | Ortho | 12.0 | Ortho | 0.42 |
| | Meta | 1.47 | Meta | 25 |
| | Para | 1.2 | Para | 13 |
| $CO_2CH_3$ | Ortho | 24 | Ortho | 35 |
| | Meta | 76 | Meta | 200 |
| | Para | 2.5 | Para | 240 |
| F | Ortho | 9.7 | Ortho | 8.3 |
| | Meta | 2.5 | Meta | 10.0 |
| | Para | 3.2 | Para | 70 |

Table 2. In vivo potency of test ligands in immature rat uterotrophic growth assay.



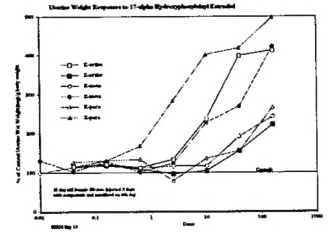


Figure 3

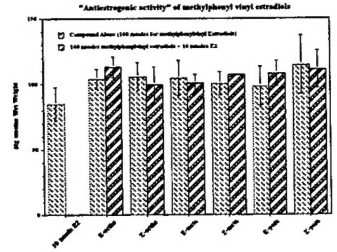


Figure 4

**Discussion:**

We have successfully applied our synthetic strategy to the preparation of several series of 17α-E- and Z-(2-,3-,4-mono-substituted-phenyl)vinyl estradiols. Although the yields were not optimized, we demonstrated the feasibility of preparing these agents by a variety of solution and solid phase methods.

We initially evaluated these compounds for their ERα-HBD binding affinity (RBA) since this value is often considered an indicator of potency. As the results in Table 1 indicate, most of the compounds retain significant affinity for the ER. In the Z-series, compounds with polar substituents, the ortho-substituted compounds had the highest RBAs, whereas, meta-substitution was favored for lipophilic groups. In the E-series, there was little discrimination except for the ortho-trifluoromethyl compound which had an RBA=223. Because all of the compounds had RBA values equal to or greater than 10 (RBA estradiol=100), the compounds were evaluated in vivo to see whether this potency profile was maintained.

As the figures for the uterotrophic growth curves indicate, all of the compounds in these series functioned as full ER agonists. Therefore, the binding of the 17α-substituents did not interfere with the activation processes downstream from ligand binding. What was of great interest to us was the significant differences in potency among the individual compounds. Potency (EC50%max) randged from 0.16 nmole for the Z-ortho-methyl compound down to 240 nmole for the E-para-methoxycarbonyl compound, over a 100-fold variation. The in vitro binding data did not provide a clear association with the in vivo growth assay. For example, the E-meta-trifluoromethyl compound had a high RBA value but low in vivo potency, whereas the Z-para-trifluoromethyl compound had a low RBA and was quite potent in vivo. Therefore, within each series it appears to be important to establish guidelines for evaluating binding-stimulation correlations.

**Summary:**

The 17α-(substituted-phenyl)vinyl estradiols are a novel class of estrogenic ligands that exhibit potent in vivo activity. The ease with which functional groups can be introduced at the distal site allows us to explore many structural variations with potential therapeutic applications. Initial screening of the compounds indicated that the ER-HBD tolerates a wide variety of functional groups at virtually any position. While the preliminary results suggest that the ligands are full estrogen receptor agonists in this assay, further testing is underway to see whether this response is maintained with other effector systems. Futher strucutral modifications may also impart partial or complete antagonism of estrogenic responses. Such studies are in progress and will be described in subsequent presentations.

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**Acknowledgments:**

This work has been supported by awards form the US PHS (1RO1-CA-81049 to RNH), USArmy (DAMD 17-99-1-9333 and 17-00-1-00384 to RNH), and the Boothroyd Foundation (to ERD). Support for the Molecular Modeling Center was provided by NSF (CHE-9974642).

# The Mitsunobu Reaction: A versatile synthetic and educational tool

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**Objectives:**

There are three objectives for the undergraduate research projects undertaken in my group.

•First, the student should gain an enhanced understanding of organic synthesis, reaction mechanisms, and the relationship between spectroscopic/physicochemical properties and molecular structure.

•Second, the student should gain practical skills in organic synthesis, isolation and purification, and spectroscopic characterization.

•Third, the student should have the opportunity to appreciate the relationship between synthetic chemistry and problem solving by participating in an ongoing research project.

**Introduction:**

One of the major areas of research in my group involves the development of therapeutic agents for the treatment of estrogen related disorders. The estrogen receptor (ER) is one member of a superfamily of nuclear receptors (NRs) that have a common structural homology and similar mechanisms of action(1,2). Because the natural estrogen-estradiol- acts as an agonist at the ER in all tissues, it mediates beneficial endocrine, central, cardiovascular and skeletal responses. It also is known to promote a number of detrimental effects, including breast cancer cell proliferation(3,4). Our research strategy has focused on preparing appropriately substituted ER-ligands to elicit selective downstream biological responses.

Over the past 5 years, several publications have appeared illustrating the interaction between estrogenic agonists and antagonists and the ER-hormone binding domain (ER-HBD)(5-7). We, as well as others have identified two classes of compounds as possessing moderate-to-high relative binding affinity (RBA) for the ER-HBD. Two examples, prepared in my laboratory (and their RBA values) are shown in Figure 1. The interaction of one, as determined by molecular modeling, is shown in Figure 2. This suggests that there exists significant steric tolerance in the region adjacent to the new aromatic ring.

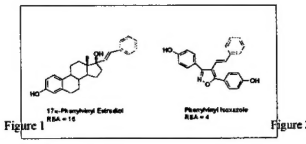



Figure 1

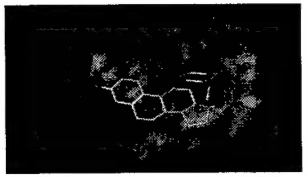


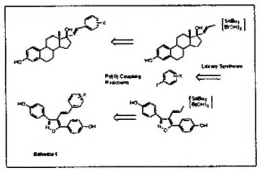
Figure 2

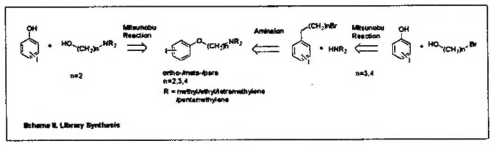
**Approach to the problem:**

The common structural feature in our led compounds is the phenylvinyl group. The strategy that we have selected for its preparation involved the versatile Pd(0) coupling reactions, exemplified by the Stille(8) and Suzuki(9) reactions. In the retrosynthetic scheme, shown below, we would be able to couple our intermediate vinyl stannane or boronic acid to the appropriate aryl iodide. Our hypothesis regarding the biological response held that the nature and position of the functional group on the aromatic ring would play a major role in the affinity, selectivity and/or efficacy of the final product. However, there was no basis a priori for knowing exactly what that functional group or its position on the aromatic ring should be. Therefore a versatile method for preparing those aryl iodides was key.

Based on previous studies, one substituent that imparted antagonist properties was the dialkylaminoalkoxyphenyl group. Although most work had utilized simple dialkyl amino groups, the ethoxy linker and para-substitution, there was no evidence from our modeling studies that this would be the optimal combination. Our target library of functionalized aryl iodides would have to include ortho-, meta-, and para-phenyl iodides, ethoxy, propoxy, and butoxy linking groups, and acyclic and cyclic amines. The synthesis of this library constituted the basis for the undergraduate research activity in my laboratory.

Although there are several methods for preparing the target dialkylaminoalkoxy aryl iodides, the most common ones [phenoxide displacement of the halogen of (haloalkyl)dialkyl amines- modified Williamson] are not applicable to all members. The Mitsunobu reaction(10,11) between a phenol and an alcohol, however, tolerates a wide variety of substitutents and utilizes readily available reagents. In addition, its reaction mechanism, its execution, and the ultimate analysis of the products provide a marvelous opportunity for students to learn about and appreciate synthetic organic chemistry.






**The Mitsunobe Reaction:**

The Mitsunobu reaction was first described in 1967(10) and has been widely used to couple many reagents, including phenols and alcohols. The reaction utilizes essentially equal molar amounts of triphenyl phosphine, diethyl (or di-isopropyl) azodicarboxylate, alcohol and phenol with slightly more than two equivalents of triethyl amine. The mechanism of the reaction is shown in Figure 3(11,12). Of importance to the students conducting the reactions, were the physico-chemical properties of the starting materials, the propertiesof the products and by-products, and the order of addition of reagents.

In our project, the aminoalcohols, where n=2, were commercially available, as were the three iodophenols. Based on safety considerations, we used the di-isopropyl azodicarboxylate DIAD. Tetrahydrofuran (THF) was freshly distilled and triethylamine (TEA) was used without further purification (13). The reaction was performed at 0-4°C for 16-24 h, the solvent was removed under reduced pressure, and the residue was purified by column chromatography, ethyl acetate-hexanes-TEA. The products were isolated and converted to their oxalate salts.

For the materials where n=3,4, the initial reaction utilized the commercially available 4-chlorobutanol or 3-bromopropanol as the alcohol component(14). In this case, TEA was not required for the chromatographic separation. The bromopropyl iodophenyl ether was the aminated with the appropriate amine to give the final product which was purified and converted to the corresponding oxalate salt.

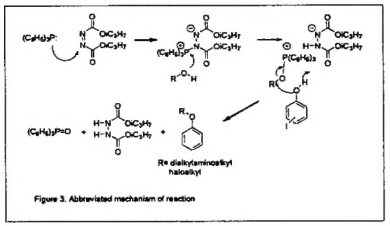



Figure 3. Abbreviated mechanism of reaction

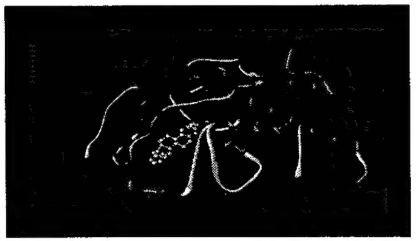


Figure 4

Results and Discussion:

The project provided the opportunity to examine a synthetically useful sequence. Although the Mitsunobu is essentially a one-steo reaction, it proceeds via several intermediates. The students need to consider the order of intermediate formation in planning the introduction of reagents. Exclusion of water is important in order to prevent competition for the reagents. Temperature control also plays a role in conducting the reaction. Consideration of the physico-chemical properties of the starting materials and products would permit the students to follow the course of the reaction, to know when it is complete and ultimately to aid in the isolation and purification process. The variety of substitution patterns require the students to predict the NMR spectra of the various products.Less obvious to the students, but of importance nonetheless, is the consideration of the scale of the reaction, whether it would be run at 1, 5 or 10mmole scale. The practical implications of work-up and subsequent uses of the materials provide pre experiment consideration.

The sequence of steps involved with the projects is as follows:

•Initial literature review of project and Mitsunobu reaction.

•Determination of reaction scale, stoichiometry, solvents, glassware, equipment.

•Consideration of reaction conditions and methods for following reaction progress.

•Reaction set-up, initiation, progress, and termination.

•Reaction work-up, isolation and purification of products.

•Characterization of reagents,products/intermediates by TLC, NMR, mp.

•Interpretation of data for each compound and comparison of series.

•Preparation of summary report describing rationale, results, significance.

Using a small library of intermediates, we were able to generate a significant range of properties. Along with these properties, the students were able to observe the effects of structure on the proton-NMR spectra. There were obvious effects with the ortho-/meta-/ para-substitution patterns, but the effects in the chemical shifts on the protons adjacent to the basic nitrogen were also instructive.

Finally, the role of the project in the overall project is instructive. Modern drug development is the utilization of many skills and disciplines. Combinatorial chemistry is a powerful tool in generating potential therapeutic agents. However, it requires the availability of the necessary reagents. In this case, one of the more advanced components of this project are working on the development of the reaction conditions for the efficient coupling of the dialkylaminoalkoxy aryl iodides to the intermediate vinyl stannanes or boronic acids. A second component is working on efficient attachment-detachment methods for solid phase synthesis, and a third component is extending biological assays to a modified high throughput assay format. The students get to see that the work they do has its industrial counterpart and will ultimately utilized in a practical application.

Conclusion:

This project has provided an educational experience for undergraduate students who are interested in organic synthesis with a bio-organic or medicinal chemistry focus. It challenges them to understand the chemistry and to apply it in a laboratory setting. The ongoing nature permits the results of the earlier work to be re-evaluated by the current students. One hopes that eventually, one of agents derived from this work will be a selective estrogen receptor agonist or antagonist (Figure 4).

**Acknowledgments**

This work has been supported in part by grants from the US PHS 1 RO1-CA-81049 and the US Army DAMD 17-00-1-00384. The Molecular Modeling Center was supported by NSF - grant CHE-9974642.

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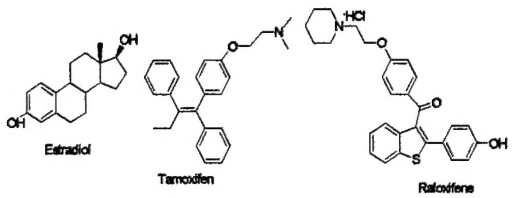
## Abstract

As part of our program to develop novel selective estrogen receptor modulator (SERMs), we chose to prepare and evaluate a series of 4-substituted-3,5-diaryl-isoxazoles. Based upon ongoing projects, we elected an approach by which the target compounds **1** could be obtained *via* palladium-catalyzed coupling reactions. In this preliminary study, Sonogashira and Stille reactions with 4-iodoisoxazole **2** were investigated to introduce alkynyl groups. The Suzuki reaction was examined by coupling **2** with phenylethenylboronic acids and by the reverse route of coupling isoxazole ethenylboronic acid **3** with aryl iodides. Synthetic and biological results will be discussed.

## Introduction

- Breast cancer is the most common cancer and the second-leading cause of cancer-related deaths in women.
- Tamoxifen, the most commonly used drug for treatment of breast cancer, is a selective estrogen receptor modulator (SERMs) that acts as an antagonist in the breast, blocking estradiol and stopping tumor growth.
- However, tamoxifen acts as an agonist in the uterus, causing increased risk of endometrial cancer.




- Raloxifene, currently used for the prevention of osteoporosis, shows promising antagonist/agonist activity w/o stimulation in the uterus.
- Tetrasubstituted pyrazoles[1] and trisubstituted isoxazoles[2] that are currently being studied also show promising results.

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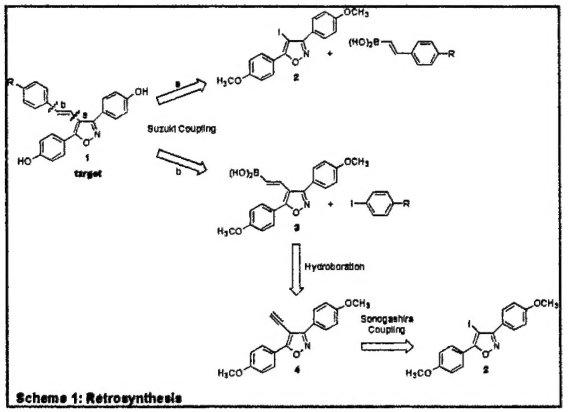
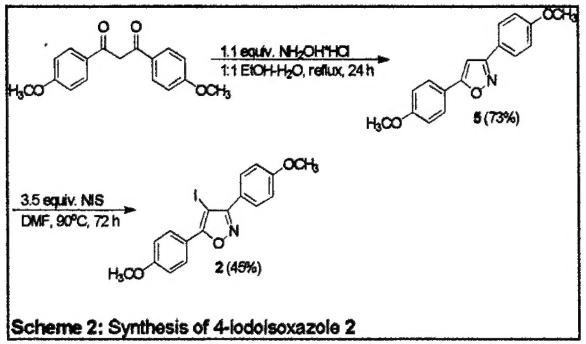
## Goals

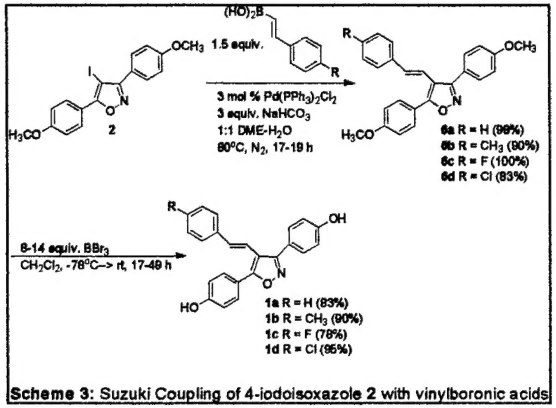
- Synthesize novel 4-E-2-(4-Rphenyl)-3,5-diarylisoxazoles **1** *via* palladium-catalyzed coupling reactions.
- Investigate the synthesis by two approaches (Scheme 1).
- Demonstrate the feasibility of these synthetic routes and the potential for future development of combinatorial libraries.




**Scheme 1: Retrosynthesis**

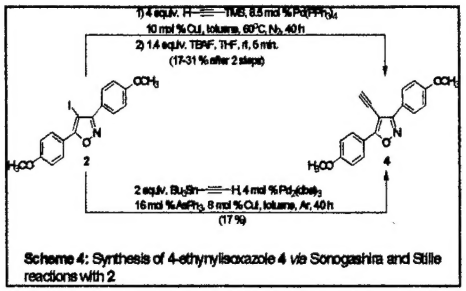



**Scheme 2: Synthesis of 4-iodoisoxazole 2**




**Scheme 3:** Suzuki Coupling of 4-iodoisoxazole **2** with vinylboronic acids

3 a) Kromann, H.; Bak, P. A.; Johansen, T. N.; Krogsgaard-Larsen, P. *Tet.* **2001**, *57*, 2195-2201. b) Lebedev, S.S.; *Syn. Commun.* **1994**, *24*, 709-719.




**Scheme 4:** Synthesis of 4-ethynylisoxazole **4** *via* Sonogashira and Stille reactions with **2**

4 a) Hoang, Y.R.; Katzenellenbogen, J.A. *Org. Lett.* **2000**, *2*, 2833-2836. b) Yamanaka, H.; Shirakura, M.; Yamamoto, E.; Sakamoto, T. *Chem. Pharm. Bull. Jpn.* **1993**, *41*, 3343-3347. c) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tet. Lett.* **1975**, 4467-4470.
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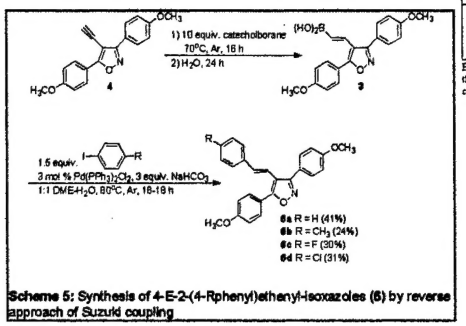


**Scheme 5:** Synthesis of 4-E-2-(4-Rphenyl)ethenyl-isoxazoles (**6**) by reverse approach of Suzuki coupling

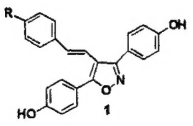


**Table 1: Relative Binding Affinities (RBA's) of 3,5-*bis*-(4-hydroxyphenyl)-4-E-2-(-Rphenyl)ethenyl-isoxazoles (1a-d)**

| Compound | R | ERα | ERβ |
|---|---|---|---|
| 1a | H | 3.1 | 0.14 |
| 1b | $CH_3$ | 2.9 | not competitive |
| 1c | F | 2.8 | 0.025 |
| 1d | Cl | 2.5 | not competitive |

Estradiol's RBA value is 100%. a) percentages were determined from an extrapolation of the expected curve. B) non competitive results were assays in which there was no competition at up to 500 nM with tritiated estradiol at 2nM.

## Results

### Chemistry

- Suzuki coupling of 4-iodoisoxazole **2** with vinylboronic acids afforded products **6a-d** in high yield.
- Sonogashira and Stille couplings of **2** gave low yields of 4-ethynylisoxazole **4**.
- Hydroboration/Suzuki coupling gave moderate conversion to **6a-d**.

### Biology

- Dihydroxy compounds **1a-d** exhibit modest binding affinity to ERα.
- However, compounds **1a-d** are highly selective for ERα over ERβ.

## Conclusion

- Route b (Scheme 1) proved to be more difficult than expected; however, route a is limited by the number of commercially available vinylboronic acids.
- Nevertheless, this study demonstrates that 4-substituted-3,5-diarylisoxazoles are accessible by the two synthetic routes featuring palladium-catalyzed coupling reactions.
- Although compounds **1a-d** show modest binding affinity, they show promising selectivity for ERα.
- Future work includes further investigation of the hydroboration/Suzuki coupling sequence to generate a larger series of derivatives for optimization.

### Acknowledgements

Army DAMD-17-00100384 PHS 1R01-CA-81049 DAMD-17-99-1-9333
Roger Kautz Jimmy Flarakos